

Environmental Issue: The overall weight of epidemiological evidence has indicated a significant association between ambient air particulate matter (PM) exposure and adverse health effects within susceptible sub-populations. Insight into the biological plausibility (PM: hazard identification, mechanisms of injury, and susceptibility) for the observed epidemiological associations with PM is still lacking and remains a critical area of research for PM risk assessment and management. Epidemiological studies have tried to determine the contribution of fine PM (PM2.5), derived from coarse (PM10-2.5), derived from natural emission sources, have on PM-associated acute mortality and morbidity. Schwartz et al. reported that PM2.5 particles not coarse particles were associated with PM acute mortality and their finding has been confirmed in a re-analysis of this data by Klemm and Masson (1.2) Schwartz et al. and Pone et al. reported that PM10-2.5 was not associated with acute PM mortality with PM2.5 (5). More recent epidemiological studies have also investigated the relative importance of fine versus coarse air particulate pollution in PM-associated acute mortality. Seven studies (2, 6 - 11) have reported greater correlations between PM2.5 and acute PM-associated mortality, whereas 4 studies found greater correlations between PM10-2.5 and acute PM-associated mortality (12 - 15). Finally, two studies have shown PM-associated morbidity correlated better with PM2.5 than for levels of PM10-2.5 (6, 16). These conflicting results suggest that geographical location, population demographics, and co-pollutant mixture effects may affect the ability of epidemiological approaches to

studies are critically needed in order to validate epidemiological based studies that have tried to link PM health effects with specific PM emission sources and atmospheric processes. Klemm RJ and Mason RM Jr. (2000). J. Air Waste Manage. Assoc. 50:1433-1439
 Schwartz J et al. (1999). Environ. Hith. Perspec. 107:339-342.

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7. Klemm RJ, et al. (2000). *J. Air Waste Manage. Assoc.* 50:1215-1222 8. Burnett RT et al. (2000). *Inhalation Toxicology* 12(suppl. 4):15-39. 9. Chock DP, Winkler S, and Chen C. (2000. J. Air Waste Manage. Assoc. 50:1481-1500.

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. Castillejos M et al. (2000). *Inhalation Toxicology* 12(suppl. 1):61-72. . Cifuentes LA et al. (2000). *J. Air Waste Manage. Assoc.* 50:1287-1298.

particulate air pollution exposure and adverse cardiovascular effects within susceptible subpopulations. The emission source(s), transformation processes, and biological mechanism(s)

unequivocally identify causal PM particles and their sources. It is clear that controlled toxicologica

that bioavailable constituents originating from the systemic delivery of pulmonary deposited combustion particles act directly on the cardiovascular system to produce adverse health effects.

The objectives of this research are to 1) identify source specific particle bioavailable constituents displaying cardiovascular toxicity; 2) identify their causal constituents and mechanism(s) of injury; 3) determine the extent to which source specific particle bioavailable constituents interact with the

cardiovascular system to influence the initiation, progression, and exacerbation of heart disease.

nental Approaches: Combustion PM: Primary fine (PM<2.5) particles were collected from the water soluble metals by ICP-AES or ICP-MS as well as for EC, OC and organic speciation. The combustion particles employed in this research have been designated; residual oil fly ash (ROFA), coal fly ash (CFA), and diesel exhaust particles (DEP).

In Vivo Toxicological Studies: Rats were exposed to ROFA by either intratracheal instillation or inhalation. Rats were exposed to DEP by intratracheal instillation. Plasma collected from ROFA exposed rats was analyzed for V and pro-inflammatory cytokines (ILβ, TNFα, IL-6 and IL-10) by ICP-AES and ELISA, respectively. Plasma collected from DEP exposed rats was analyzed for benzo-a-pyrene and related metabolites by HPLC. RT-PCR analysis was employed to assess gene expression alterations in RNA recovered from hearts taken form control and combustion PM-exposed rats. Kinase immunopreciptation and Western blot analyses were employed to assess alterations in cardiac intracellular signaling pathways in protein extracted from hearts recovered form control and

<u>Ex Vivo Organ Toxicological Studies</u>: Langendorff perfusion of rat hearts was performed as described by Gabel et al. (Am. J. Physiol. (2001) 280:H1963-H1969). Functional end points such as heart rate, left ventricular developed pressure (LVDP), perfusion flow rate (FR), arrhythmia frequency (AF) were measured. Alterations in high energy phosphate metabolites were continually monitored by ³¹P NMR. Aliquots of the perfusate were analyzed for lactate dehydrogenase and creatine kinase enzyme activity. Aortic rings recovered from lean healthy (cp/?) or obese type 2 diabetic (JCR:LA-cp) rats were exposed ex vivo to ROFA-L as described by Russell et al. (J. Pharmacol. Exp. Ther. (2000) 295:753-760). The effects which ROFA-L had on phenylephrine induced vasoconstriction and acetylcholine induced

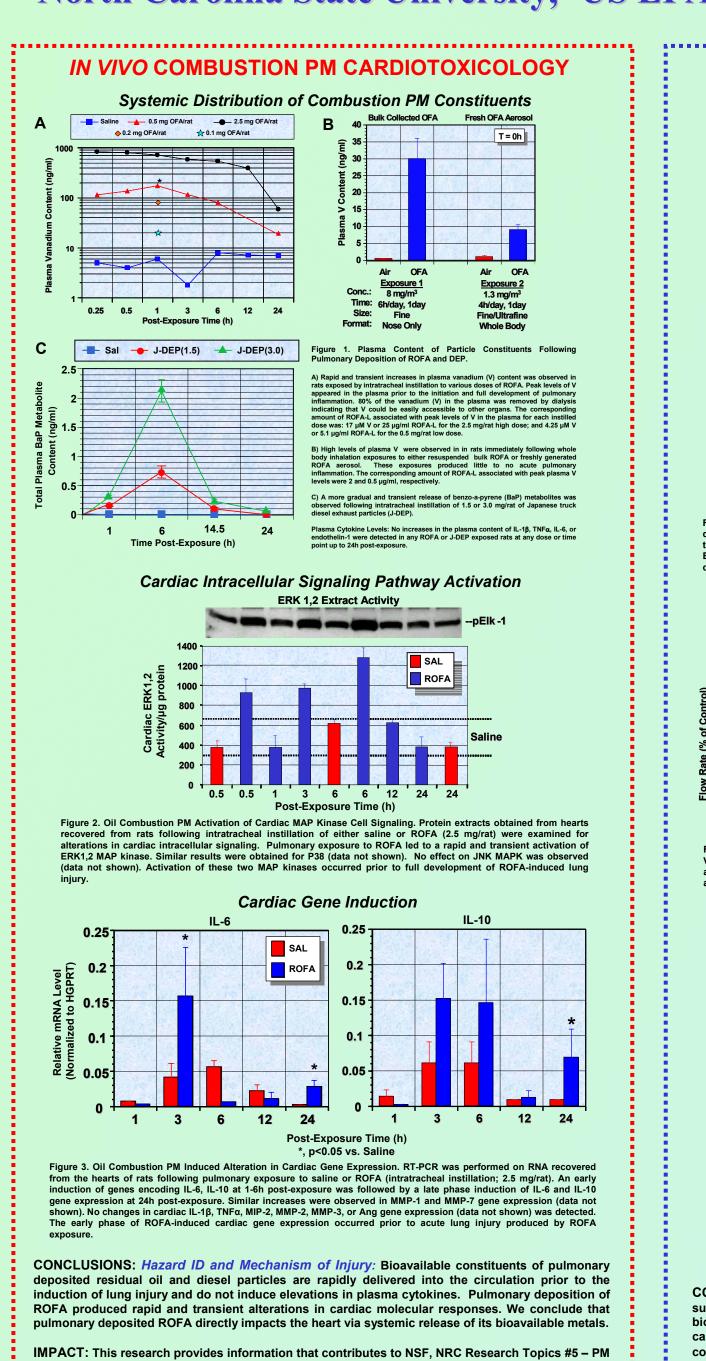
In Vitro Celluar Toxicological Studies: Primary cultures of cardiomyocytes (CM) and cardiofibroblasts (CF) were prepared from 1 day old, Sprague Dawley, rats. Primary CM and CF cultures were exposed to aqueous (saline) or organic (dimethylsulfoxide) extracts of various combustion particles as well as various sub-fractions of these extracts. Cellular cytotoxicity was monitored by percent of total lactate dehydrogenase (LDH) release. Alteration in the spontaneous beating frequency was monitored in order to determine the ability of combustion PM aqueous and organic extracts to alter CM function. RT-PCR analysis was employed to assess gene expression alterations in RNA recovered from CMs or CFs following exposure to aqueous extracts of ROFA. Kinase immunopreciptation and Western blot analyses were employed to assess alterations in intracellular signaling pathways in protein extracted

from CFs following exposure to particle free saline leachate of ROFA.



PARTICULATE MATTER Cardiovascular Toxicity of Primary Combustion Particles: Linking Adverse Health Effects to Sources

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Hazard Identification and #9 - PM Toxicity Mechanisms. Research that identifies what primary

emission particles, and their physicochemical properties, that are most capable of adversely

FUTURE DIRECTIONS: Near Term Research - will assess the ability of pulmonary deposited

combustion particles (diesel and coal) to alter cardiovascular (CV) cellular and molecular endpoints

CV responses with combustion PM physicochemical properties. Long Term Research - will

employ functional genomics, proteomics, and microdissection technologies to assess in cardiac

compromised rodents the ability of various combustion source PMs to: 1) influence the progression

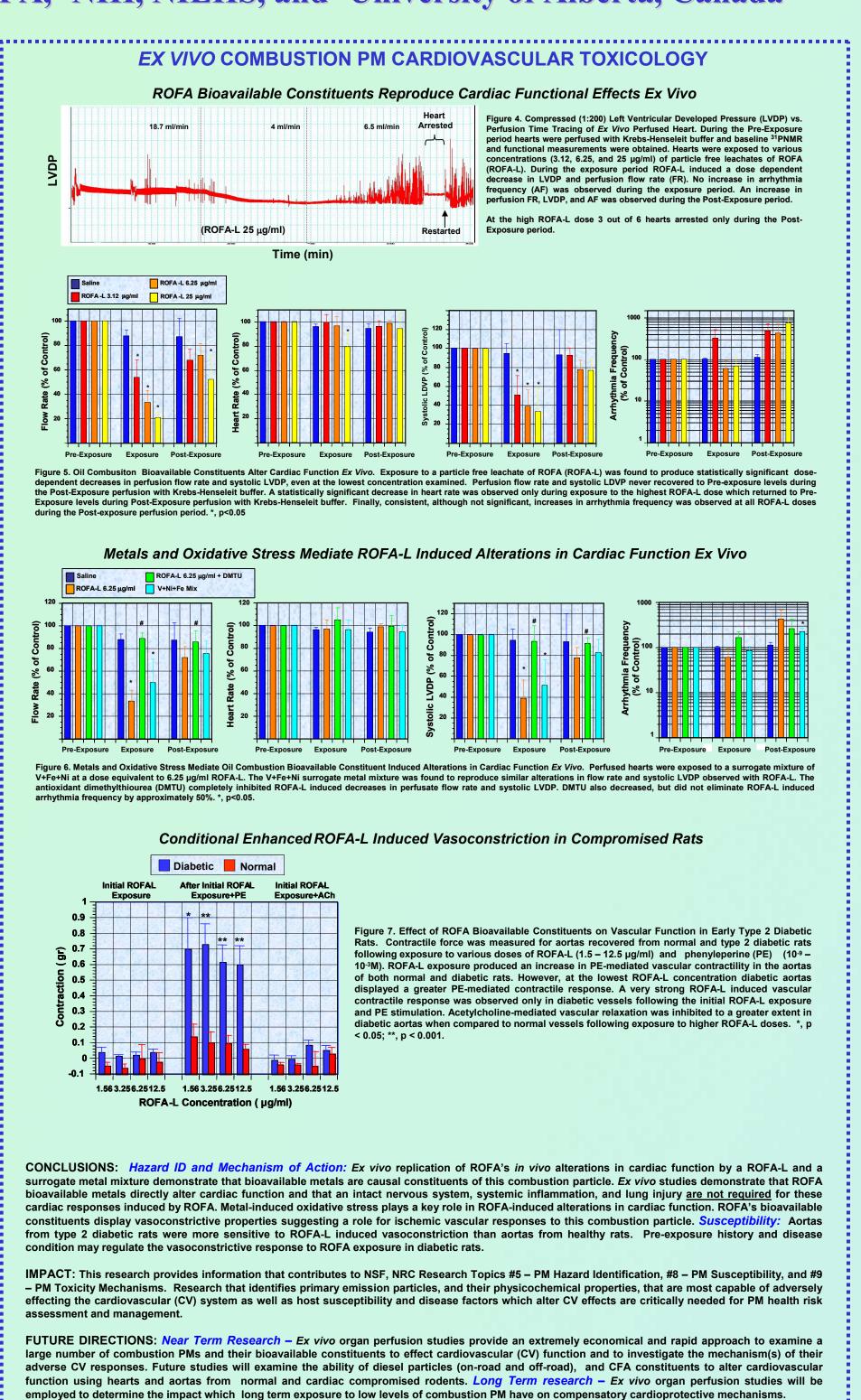
and exacerbation of CV disease; 2) correlate effects with PM physicochemical properties; and 3)

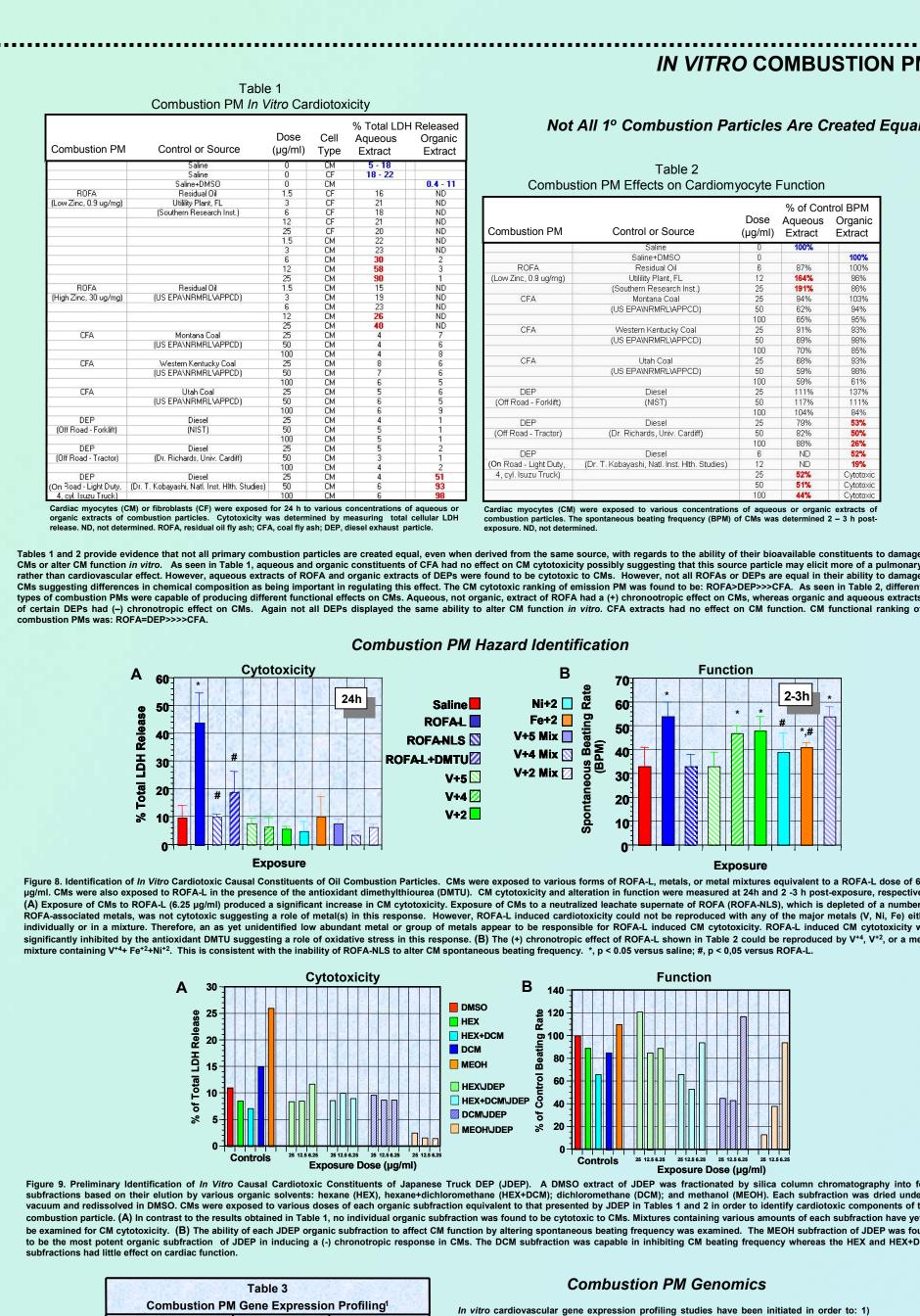
identify the underlying molecular pathology and susceptibility associated with any observed effects.

in healthy and cardiac compromised rodents. Particular attention will focus on correlating adverse

health effects. Such information is critically for PM health risk assessment and management.

effecting the cardiovascular system will provide information that will link specific sources to adverse





- Intracellular Signaling

- Functionally

Unclassified³

46 out of 3924

540 out of 3924

.5 μg/ml for 1 hour prior to RNA isolation. Gene expression studies were done using

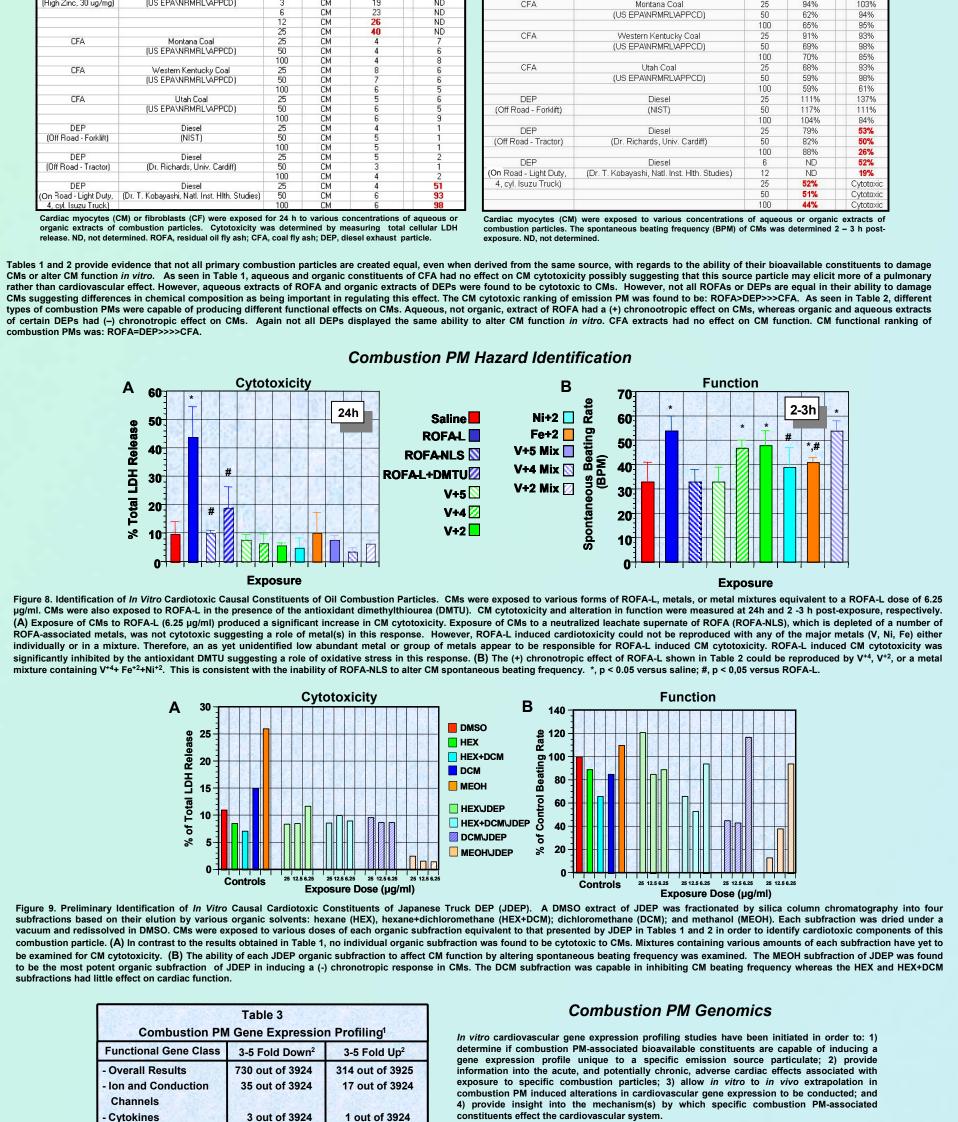
2) Number of genes in each functional class that were either up or down regulated out

of the total number of genes assayed on the microarray.
3) 2172 out of 3924 genes on the microarray are listed in the Functionally Unclassified

oled saline or ROFA-L samples derived from 5 separate CM cultures

10 out of 3924

235 out of 3924



IN VITRO COMBUSTION PM CARDIOTOXICOLOGY

Dose Aqueous Organ

(ug/ml) Extract Extra

Saline+DMSO Residual Oil Utilility Plant, FL

Preliminary results of ROFA-L induced alterations in cardiomyocyte gene expression is shown

in Table 3. The expression of 1044 out of 3924 genes (27%) were altered following an acute

exposure to bioavailable constituents derived from oil combustion particles. Results from

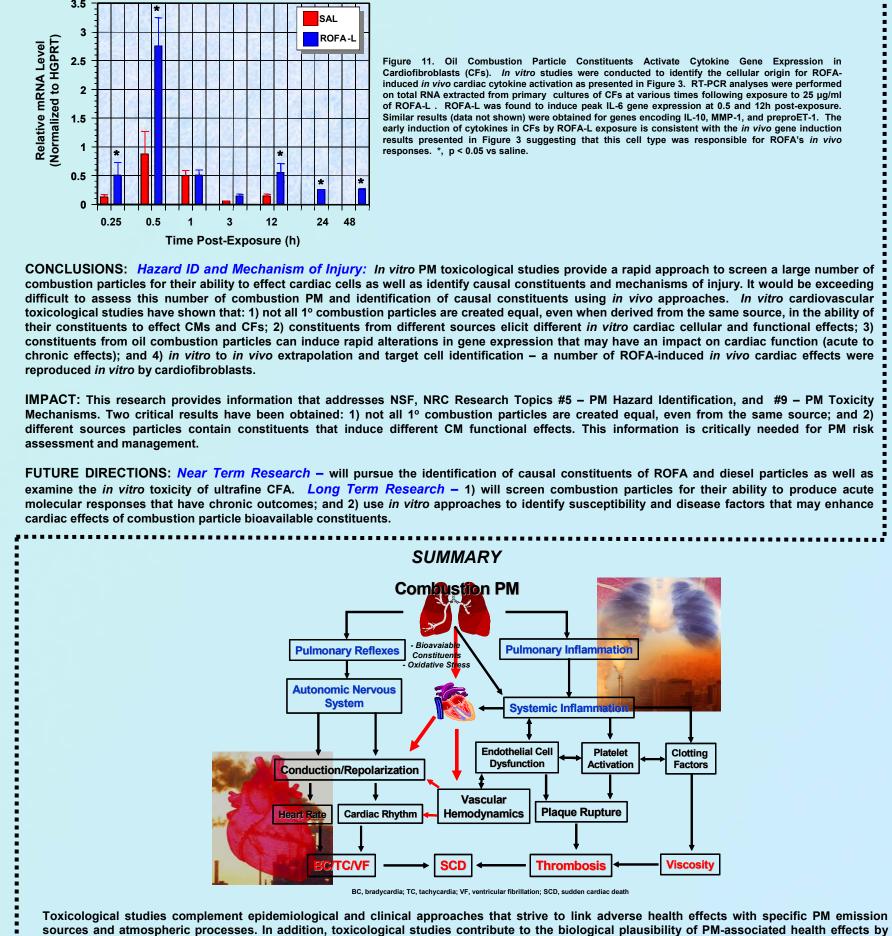
initial bioinformatics analysis of the data showed significant alterations in genes that

regulated cardiac cell growth and function such as intracellular signaling pathway

associated cell growth\survival, ion channel and gap junction proteins which regulate

electrical repolarization and conduction. Very few alterations in cytokine gene expression was

observed following an acute exposure to a low dose of ROFA-L.



providing insight into the mechanisms of injury and elucidating the underlying molecular susceptibilities associated with adverse PM health

responses. As shown above, pulmonary deposited particles can adversely effect the cardiovascular (CV) system by a variety of mechanisms. This

research has employed new and alternative ex vivo and in vitro approaches that can rapidly and economically examine a large number of

combustion source particles, and their constituents, for adverse CV effects. Bioavailable constituents of combustion particles derived from

specific sources directly and adversely effect (red arrows in figure) the heart at the functional, cellular, and molecular levels. Oxidative stress plays

a key role in many of the combustion particle induced adverse CV responses. A critical finding was that not all combustion source particles, even

those derived from the same source, are created equal in their ability to induced CV effects highlighting the importance for source PM hazard ID as

well as conditions of generation. Ultimately this research will provide a database to link adverse ambient air PM CV effects with specific

combustion sources.

1h Post-Exposure

Figure 10. Oil Combustion Particle Constituents Activate Cardiofibroblast MAP Kinase Intracellular Signaling. In vitro studies were conducted to identify the cellular origin for ROFA-induced in vivo cardiac ERK1,2 kinase activation as presented in Figure 2. Dually phosphorylated (p)ERK1,2 Western blot analysis was performed on protein extracts recovered from primary

cultures of cardiac fibroblasts (CFs), derived from 1 day old rats, following a 1h exposure to various concentrations of ROFA-L. (A) ROFA-L produced a dose-response increase in the

or these metals in this response. The antioxidant DMTU completely inhibited ROFA-L induction of pERK1,2 levels in CFs demonstrating a role of oxidative stress in ERK1,2 activation. (C) A

Target Cell Identification

25 μg/ml, 1h

25 μg/ml, 1h

SOLVING AGENCY PROBLEMS